Department _____

Subject _____

Name ___ (Tiao-Liang / Yu  π

Address _____

National®Brand
# Computation Notebook
11³/₄" x 9¹/₄", 4 x 4 Quad., 75 Sheets    **43-648**

0  73333 43648  8

**AVERY DENNISON**

Office Products
Chicopee, MA 01022

Department _____

Subject _____

Name _Guo-Liang Yu II_

Address _____

National®Brand

# Computation Notebook

11³/₄" x 9¹/₄", 4 x 4 Quad.. 75 Sheets    **43-648**

0  73333 43648  8

AVERY
DENNISON

Office Products
Chicopee. MA  01022

1/23/96   Large scale plasmid prep for cDNA library

HBC   to be used to make single stranded DNA

to test SAGE

— transformation from an old stock of plasmid DNA

— obtain titer  $1.5 \times 10^{7}$ cfu   grow up to 1 L o/n

— plasmid prep

  spin down, resuspend in 40ml $H_2O$

  add                          8ml  NaOH/SDS

                               60ml 3M KOAc

  Spin down   ppt with isopropanol

  CsCl₂ gradient      60K o/N hr

  concentration of DNA is low

1/24 proliferation assay for endothelial cells

EDAP

Cell lines    Arterial endothelial cell

Veinus endothelial cells

Huvec

Seed $5 \times 10^3$ cells to each 96 well    DMEM 10% FBS

5 hr incubation — Let cell attach, remove medium

add samples as show below

|  | TNFα | | | | EDAP E2 | | | | EDAP E3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
|  | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
| A | / | 1 ug | | | 1 us | | | | 1 us | | 10% | |
| B | / | 100 ng | | | 100 ng | | | | 100 ng | | 1% | |
| C | / | 10 ns | | | 10 ns | | | | 10 ns | | 0.1% | |
| D | / | 1 ng | | | 1 ng | | | | 1 ng | | 0.1% | |
| E | / | bfr | | | 1 us | | | | 100 ng | | 10% | |
| F | / | | | | 100 ns | | | | 10 ng | | 1% | |
| G | / | Control | | | 10 ns | | | | 1 ng | | 0.1% | |
| H | / | | | | 1 ng | | | | 0.1 ng | | 0.1% | |

EDAP E4    bFGF

40 hr after PMTS assay for AE & VE

add $^3H$ thymidine

incubate 6 hrs

wash once w/ PBS.    Lyse cell    Count

## Certificate of Analysis

Protein Name:  TNF gamma (EDAP)

Batch #: HG02704-E2                                    Date: 11/10/95

Expression System: E. Coli

Method of Purification:
1, Cell disruption and purification of inclusion body
Wash pellet with TE, 1M, 2 M Urea, and
2, Extraction TNF γ with  4 and 8 M Urea
3, Buffer exchange (2XPBS) and refolding in PD-10
4, Removing endotoxin by affinity gel chromatography

Estimated Purity: > 80%


— TNF gamma

Protein Concentration:

Method A: 176 µg/ml

Method B: 180 µg/ml

Endotoxin level in sample: 0.17 EU/mg

Method of Analysis: Limulus Amebocyte Lysate test  (Bio-Whittaker)

Sterile filtered:   Yes __X__  No ___
Buffer composition: 2XPBS
Total amount of protein purified: 14 mg
Prepared by:Jian NI

Approved by:  _(signature)_

_(handwritten: 1.5 md  SB  10/30)_

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## Certificate of Analysis

Protein Name:  TNF gamma
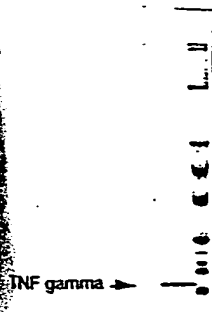
Batch #: HG02703-3E        _A3   5/25 ~ 5/26._  Date: 5/26

Expression System: E. Coli

Method of Purification:

1, Cell disruption and purification of inclusion body
2, Solubilization of protein with 8 M urea
3, Sephadex S-200 size exclusion chromatography
4, Dialysis into PBS

Estimated Purity: > 70%


TNF gamma →

Protein Concentration: 80 µg/ml

Endotoxin level in sample: 525 EU/mg

Method of Analysis: Limulus Amebocyte Lysate test (Bio-Whittaker)

Buffer composition: PBS

Endotoxin level in buffer: <10 EU/ml

Sterile filtered:   Yes XXX No ___

Prepared by:  Jian NI

Approved by:  _(signature)_

# Certificate of Analysis

**Protein Name:** TNF-delta (His-tag)
**Clone ID#:** HLTBT71

**Batch #:HG10700-E1**

Date:1/22/96

**Expression System:** E. Coli

**Molecular weight:** 20.8 Kd

**Method of Purification:**
Nickel-chelate affinity chromotography column
Buffer exchange (2XPBS) and refolding in PD-10 column

**Estimated Purity:** 80%

**Protein Concentration:**

Method A: 200 µg/ml

Method B: 160 µg/ml

**Endotoxin level in sample:** 2.8 EU/mg

**Method of Analysis:** Limulus Amebocyte Lysate test  (Bio-Whittaker

**Sterile filtered:**  Yes __Y_  No __

**Buffer composition:** 2XPBS

**Biological activity**
    yes/not tested

**Total amount of protein purified:** 10 mg

**Prepared by:**  Jian Ni

**Approved by:**

Updated September 15, 1995

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# Certificate of Analysis

in Name:  TNF gamma (EDAP)

#: HG02704-E2

Date: 11/10/95

ssion System: E. Coli

d of Purification:
 disruption and purification of inclusion body
 ellet with TE, 1M, 2 M Urea, and
 ction TNF γ with  4 and 8 M Urea
 r exchange (2XPBS) and refolding in PD-10
 oving endotoxin by affinity gel chromatography

ted Purity: > 80%

TNF gamma

Concentration:

hod A: 176 µg/ml

hod B: 180 µg/ml

level in sample: 0.17 EU/mg

Analysis: Limulus Amebocyte Lysate test  (Bio-Whittaker)

---

size exclusion chromatography
4. Dialysis into PBS

**Estimated Purity:** > 70%

TNF gamma ➤

**Protein Concentration:** 80 µg/ml

**Endotoxin level in sample:** 525 EU/mg

**Method of Analysis:** Limulus Amebocyte Lysate test (Bio-Whittaker)

**Buffer composition:** PBS

**Endotoxin level in buffer:** <10 EU/ml

**Sterile filtered:**  Yes XXX No __

**Prepared by:**  Jian Ni

**Approved by:**

Updated May 23, 1995

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# Certificate of Analysis

**Protein Name:** TNF-gamma (EDAP) (His-tag)
**Clone ID#:** HUVEO91

**Batch #:HG02702-E4**

Date:1/22/96

**Expression System:** E. Coli

**Molecular weight:** 20.1 Kd

**Method of Purification:**
Nickel-chelate affinity chromotography column
Buffer exchange (2XPBS) and refolding in PD-10 column

**Estimated Purity:** >80%
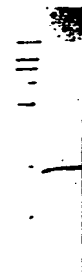
**Protein Concentration:**

Method A: 150 µg/ml

Method B: 260 µg/ml

**Endotoxin level in sample:** 3.1 EU/mg

**Method of Analysis:** Limulus Amebocyte Lysate test  (Bio-Whittaker

**Sterile filtered:**  Yes __Y_  No __
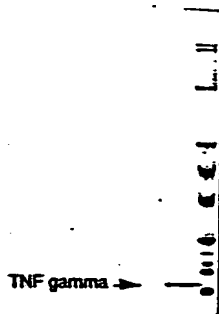
**Buffer composition:** 2XPBS

**Biological activity**
    yes/not tested

**Total amount of protein purified:** 10 mg

**Prepared by:**  Jian Ni

**Approved by:**

Updated September 15, 1995

MTS assay:

AE .

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| A | | 0.019 | 0.033 | 0.070 | 0.033 | 0.026 | 0.013 | 0.070 | 0 | 0.012 | 0.241 | |
| B | | 0.47 | 0.043 | 0.71 | 0.058 | 0.050 | 0.043 | 0.047 | 0.42 | 0.28 | 0.162 | |
| C | | 0.039 | 0.04 | 0.73 | 0.040 | 0 | 0 | 0.048 | 0.08 | -0.025 | 0.028 | |
| D | | -0.051 | 0.04 | 0.020 | 0.014 | 0 | 0 | 0 | -0.04 | -0.02 | 0 | |
| E | | -0.02 | 0.038 | 0.043 | 0.010 | -0.02 | 0.017 | 0.082 | 0.77 | 0.070 | 0.289 | |
| F | | -0.02 | 0.066 | 0.045 | 0.044 | 0.033 | 0.04 | 0.145 | 0.133 | 0.14 | 0.212 | |
| G | | 0.015 | 0.04 | 0.028 | 0.02 | 0.07 | 0 | 0.86 | 0.091 | 0.1 | 0.05 | |
| H | | -0.01 | 0.061 | 0.012 | 0.092 | 0.039 | 0.01 | 0.642 | 0.013 | 0.041 | 0.01 | |

by            EGF            BFGF        FBS

VB

| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **A** Limit Absorb Range | -0.005 | -0.013 | -0.008 | 0.007 | 0.039 | 0.050 | 0.021 | 0.017 | 0.001 | -0.004 | 0.658 | -0.020 |
| **B** Limit Absorb Range | -0.034 | -0.040 | -0.008 | 0.003 | 0.008 | 0.030 | 0.024 | 0.021 | 0.019 | 0.023 | 0.688 | -0.013 |
| **C** Limit Absorb Range | -0.028 | -0.027 | -0.007 | -0.010 | 0.027 | 0.027 | 0.024 | 0.035 | 0.096 | 0.040 | 0.124 | -0.008 |
| **D** Limit Absorb Range | -0.025 | -0.003 | 0.010 | 0.015 | 0.023 | 0.028 | 0.026 | 0.038 | 0.024 | 0.034 | 0.058 | -0.002 |
| **E** Limit Absorb Range | -0.024 | -0.014 | -0.005 | -0.016 | 0.013 | 0.014 | 0.016 | 0.309 | 0.237 | 0.269 | 0.847 | 0.000 |
| **F** Limit Absorb Range | -0.074 | -0.060 | -0.035 | -0.032 | -0.030 | -0.008 | 0.011 | 0.382 | 0.331 | 0.395 | 0.646 | -0.013 |
| **G** Limit Absorb Range | -0.042 | -0.022 | -0.014 | -0.018 | 0.002 | -0.013 | 0.009 | 0.171 | 0.191 | 0.213 | 0.158 | -0.006 |
| **H** Limit Absorb Range | -0.027 | -0.025 | -0.018 | 0.005 | 0.018 | 0.019 | 0.004 | 0.090 | 0.094 | 0.069 | 0.042 | 0.000 |

Huvec  AE

TUE 15 H

```
USER: 3 ID:3H CPM      PRESET TIME: 1.00
SAMPLE REPEAT: 1 CYCLE REPEAT: 1 SCR:N  RS232:N
H#: 1 AOC:N QCF:N RCM:Y
RCM-TIME: 0.50 INT:999.95
CHANNEL 1-LL: 0 UL: 400 2SIGMA: 0.50 BKG SUB:   0.00 BKG 2SIG
DATA CALC: CPM, UNKNOWN REPLICATES: 1  NORM FACTOR:Q 1.00000
HALF LIFE(DAYS):N
```
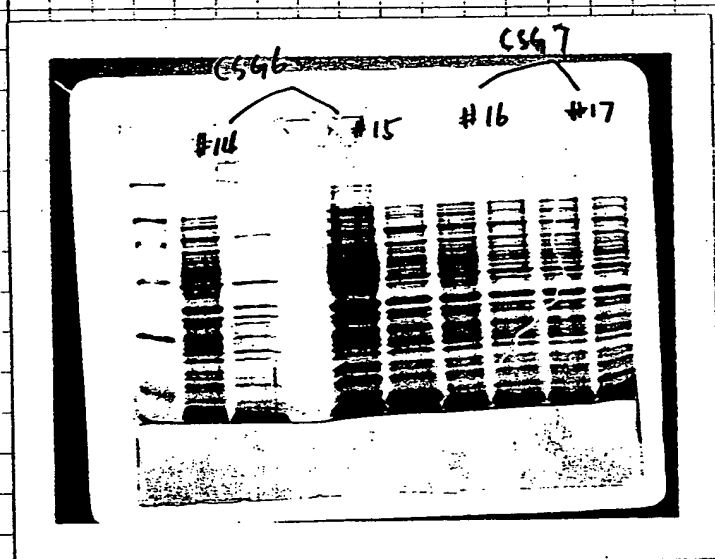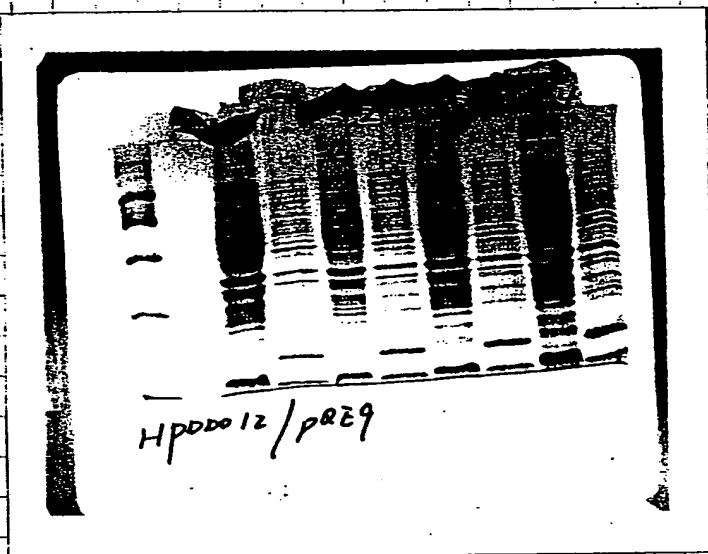
| SAM | POS | CH | CPM | 2SIG% | TIME | EL TIME | AVG H# | RCM% |
|---|---|---|---|---|---|---|---|---|
| 1 | 163 | 1 | 253.00 | 12.57 | 1.00 | 1.19 | 62.0 | 18.31 |
| 2 | 164 | 1 | 1552.00 | 5.08 | 1.00 | 2.42 | 61.0 | 1.30 |
| 3 | 165 | 1 | 141.00 | 16.84 | 1.00 | 3.67 | 64.0 | 21.97 |
| 4 | 166 | 1 | 301.00 | 11.53 | 1.00 | 4.90 | 66.0 | 18.88 |
| 5 | 167 | 1 | 397.00 | 10.04 | 1.00 | 6.14 | 64.0 | 10.93 |
| 6 | 168 | 1 | 223.00 | 13.39 | 1.00 | 7.38 | 64.0 | 21.35 |
| 7 | 169 | 1 | 365.00 | 10.47 | 1.00 | 8.61 | 63.0 | 11.71 |
| 8 | 170 | 1 | 336.00 | 10.91 | 1.00 | 9.85 | 64.0 | 23.55 |
| 9 | 171 | 1 | 186.00 | 14.66 | 1.00 | 11.10 | 60.0 | 12.63 |
| 10 | 172 | 1 | 116.00 | 18.57 | 1.00 | 12.34 | 63.0 | 55.25 |
| 11 | 173 | 1 | 266.00 | 12.26 | 1.00 | 13.57 | 62.0 | 14.96 |
| 12 | 174 | 1 | 635.00 | 7.94 | 1.00 | 14.80 | 64.0 | 3.46 |
| 13 | 175 | 1 | 98.00 | 20.20 | 1.00 | 16.04 | 63.0 | 18.38 |
| 14 | 176 | 1 | 253.00 | 13.10 | 1.00 | 17.28 | 70.0 | 32.91 |
| 15 | 177 | 1 | 164.00 | 15.62 | 1.00 | 18.52 | 63.0 | 16.88 |
| 16 | 178 | 1 | 56.00 | 26.73 | 1.00 | 19.74 | 57.0 | 20.00 |
| 17 | 179 | 1 | 112.00 | 18.90 | 1.00 | 20.97 | 60.0 | 14.87 |
| 18 | 180 | 1 | 101.00 | 19.90 | 1.00 | 22.21 | 59.0 | 15.98 |
| 19 | 181 | 1 | 1932.00 | 4.55 | 1.00 | 23.44 | 59.0 | 1.18 |
| 20 | 182 | 1 | 962.00 | 6.45 | 1.00 | 24.67 | 61.0 | 3.14 |
| 21 | 183 | 1 | 397.00 | 10.59 | 1.00 | 25.92 | 64.0 | 27.14 |
| 22 | 184 | 1 | 195.00 | 14.32 | 1.00 | 27.16 | 67.0 | 38.97 |
| 23 | 185 | 1 | 687.00 | 7.63 | 1.00 | 28.40 | 67.0 | 8.14 |
| 24 | 186 | 1 | 354.00 | 10.63 | 1.00 | 29.63 | 63.0 | 14.77 |
| 25 | 187 | 1 | 177.00 | 15.03 | 1.00 | 30.88 | 65.0 | 12.05 |
| 26 | 188 | 1 | 261.00 | 12.38 | 1.00 | 32.13 | 66.0 | 27.81 |
| 27 | 189 | 1 | 248.00 | 12.70 | 1.00 | 33.36 | 69.0 | 19.46 |
| 28 | 190 | 1 | 238.00 | 12.96 | 1.00 | 34.59 | 64.0 | 5.22 |
| 29 | 191 | 1 | 477.00 | 9.16 | 1.00 | 35.84 | 66.0 | 16.67 |
| 30 | 192 | 1 | 325.00 | 11.09 | 1.00 | 37.08 | 67.0 | 23.74 |
| 31 | 193 | 1 | 357.00 | 10.59 | 1.00 | 38.31 | 66.0 | 8.05 |
| 32 | 194 | 1 | 366.00 | 10.45 | 1.00 | 39.56 | 66.0 | 27.29 |
| 33 | 195 | 1 | 351.00 | 10.68 | 1.00 | 40.80 | 65.0 | 22.51 |
| 34 | 196 | 1 | 242.00 | 12.66 | 1.00 | 42.03 | 65.0 | 7.28 |
| 35 | 197 | 1 | 325.00 | 11.09 | 1.00 | 43.28 | 65.0 | 31.23 |
| 36 | 198 | 1 | 436.00 | 9.58 | 1.00 | 44.53 | 64.0 | 24.16 |
| 37 | 199 | 1 | 473.00 | 9.20 | 1.00 | 45.77 | 70.0 | 6.82 |
| 38 | 200 | 1 | 428.00 | 9.67 | 1.00 | 47.00 | 66.0 | 8.95 |
| 39 | 201 | 1 | 297.00 | 11.61 | 1.00 | 48.23 | 69.0 | 10.21 |
| 40 | 202 | 1 | 318.00 | 11.22 | 1.00 | 49.48 | 68.0 | 30.35 |
| 41 | 203 | 1 | 348.00 | 10.72 | 1.00 | 50.73 | 70.0 | 25.16 |
| 42 | 204 | 1 | 231.00 | 13.16 | 1.00 | 51.97 | 65.0 | 33.15 |
| 43 | 205 | 1 | 394.00 | 10.08 | 1.00 | 53.22 | 67.0 | 25.10 |
| 44 | 206 | 1 | 892.00 | 6.70 | 1.00 | 54.47 | 70.0 | 13.91 |
| 45 | 207 | 1 | 115.00 | 18.65 | 1.00 | 55.71 | 67.0 | 61.78 |
| 46 | 208 | 1 | 1239.00 | 5.68 | 1.00 | 56.96 | 68.0 | 6.30 |
| 47 | 209 | 1 | 553.00 | 8.50 | 1.00 | 58.20 | 67.0 | 13.58 |
| 48 | 210 | 1 | 244.00 | 12.80 | 1.00 | 59.45 | 71.0 | 23.42 |
| 49 | 211 | 1 | 266.00 | 12.26 | 1.00 | 60.69 | 67.0 | 15.31 |
| 50 | 212 | 1 | 435.00 | 9.59 | 1.00 | 61.92 | 69.0 | 5.15 |
| 51 | 213 | 1 | 289.00 | 11.76 | 1.00 | 63.16 | 64.0 | 19.52 |
| 52 | 214 | 1 | 197.00 | 14.25 | 1.00 | 64.50 | 69.0 | 13.84 |
| 53 | 215 | 1 | 243.00 | 12.83 | 1.00 | 65.74 | 67.0 | 14.80 |
| 54 | 216 | 1 | 333.00 | 10.96 | 1.00 | 66.99 | 67.0 | 29.94 |
| 55 | 217 | 1 | 143.00 | 16.72 | 1.00 | 68.23 | 68.0 | 34.64 |
| 56 | 218 | 1 | 183.00 | 14.78 | 1.00 | 69.46 | 67.0 | 17.74 |
| 57 | 219 | 1 | 6583.00 | 2.47 | 1.00 | 70.71 | 67.0 | 1.09 |
| 58 | 220 | 1 | 907.00 | 6.64 | 1.00 | 71.94 | 64.0 | 3.70 |
| 59 | 221 | 1 | 470.00 | 9.23 | 1.00 | 73.18 | 64.0 | 6.05 |
| 60 | 222 | 1 | 335.00 | 10.93 | 1.00 | 74.42 | 64.0 | 12.91 |
| 61 | 223 | 1 | 303.00 | 11.49 | 1.00 | 75.65 | 64.0 | 7.33 |
| 62 | 224 | 1 | | | | 73.90 | | |
| 63 | 225 | 1 | 267.00 | 12.24 | 1.00 | 77.13 | 61.0 | 7.40 |
| 64 | 226 | 1 | 307.00 | 11.41 | 1.00 | 78.36 | 66.0 | 9.40 |
| 65 | 227 | 1 | 369.00 | 10.41 | 1.00 | 79.61 | 65.0 | 20.69 |
| 66 | 228 | 1 | 404.00 | 9.95 | 1.00 | 80.85 | 68.0 | 16.84 |
| 67 | 229 | 1 | 237.00 | 12.99 | 1.00 | 82.07 | 63.0 | 9.55 |
| 68 | 230 | 1 | 230.00 | 12.65 | 1.00 | 83.32 | 63.0 | 14.46 |
| 69 | 231 | 1 | 336.00 | 10.91 | 1.00 | 84.55 | 66.0 | 7.24 |
| 70 | 232 | 1 | 217.00 | 13.58 | 1.00 | 85.78 | 64.0 | 14.92 |
| 71 | 233 | 1 | 924.00 | 6.58 | 1.00 | 87.02 | 66.0 | 7.37 |
| 72 | 234 | 1 | 264.00 | 12.31 | 1.00 | 88.36 | 69.0 | 4.91 |
| 73 | 235 | 1 | 769.00 | 7.12 | 1.00 | 89.59 | 63.0 | 3.62 |
| 74 | 236 | 1 | 1448.00 | 5.26 | 1.00 | 90.84 | 67.0 | 6.21 |
| 75 | 237 | 1 | 651.00 | 7.84 | 1.00 | 92.08 | 68.0 | 8.77 |
| 76 | 238 | 1 | 832.00 | 6.93 | 1.00 | 93.32 | 67.0 | 22.56 |
| 77 | 239 | 1 | 534.00 | 10.21 | 1.00 | 94.57 | 67.0 | 5.44 |
| 78 | 240 | 1 | 326.00 | 11.08 | 1.00 | 95.91 | 69.0 | 15.56 |
| 79 | 241 | 1 | 349.00 | 10.71 | 1.00 | 97.15 | 68.0 | 13.21 |
| 80 | 242 | 1 | 258.00 | 12.72 | 1.00 | 98.39 | 63.0 | |

| SAM | POS | CH | CPM | 2SIG% | TIME | EL TIME | AVG H# | RCM% |
|---|---|---|---|---|---|---|---|---|
| 81 | 243 | 1 | 340.00 | 13.85 | 1.00 | 99.62 | 59.0 | 12.02 |
| 82 | 244 | 1 | 82.00 | 22.09 | 1.00 | 100.86 | 59.0 | 46.70 |
| 83 | 245 | 1 | 70.00 | 23.90 | 1.00 | 102.09 | 54.0 | 46.85 |
| 84 | 246 | 1 | 584.00 | 8.28 | 1.00 | 103.34 | 52.0 | 5.79 |
| 85 | 247 | 1 | 65.00 | 25.20 | 1.00 | 104.57 | 51.0 | 37.26 |
| 86 | 248 | 1 | 87.00 | 21.44 | 1.00 | 105.81 | 51.0 | 53.49 |
| 87 | 249 | 1 | 175.00 | 15.12 | 1.00 | 107.04 | 59.0 | 19.57 |
| 88 | 250 | 1 | 76.00 | 22.94 | 1.00 | 108.28 | 55.0 | 53.38 |
| 89 | 251 | 1 | 59.00 | 26.04 | 1.00 | 109.52 | 53.0 | 33.55 |
| 90 | 252 | 1 | 70.00 | 23.90 | 1.00 | 110.75 | 53.0 | 39.26 |
| 91 | 253 | 1 | 50.00 | 28.28 | 1.00 | 111.98 | 52.0 | 39.88 |
| 92 | 254 | 1 | 41.00 | 31.23 | 1.00 | 113.22 | 52.0 | 44.14 |
| 93 | 255 | 1 | 44.00 | 30.15 | 1.00 | 114.45 | 51.0 | 23.34 |
| 94 | 256 | 1 | 44.00 | 30.15 | 1.00 | 115.68 | 52.0 | 39.68 |
| 95 | 257 | 1 | 147.00 | 16.50 | 1.00 | 118.17 | 52.0 | 33.02 |
| 96 | 258 | 1 | 56.00 | 26.73 | 1.00 | 119.40 | 50.0 | 36.20 |
| 97 | 259 | 1 | 52.00 | 27.74 | 1.00 | 120.64 | 55.0 | 51.11 |
| 98 | 260 | 1 | 108.00 | 19.25 | 1.00 | 121.87 | 52.0 | 29.10 |
| 99 | 261 | 1 | 163.00 | 15.67 | 1.00 | 123.11 | 51.0 | 23.20 |
| 100 | 262 | 1 | 55.00 | 27.47 | 1.00 | | | |
| 101 | 263 | 1 | 55.00 | 26.97 | 1.00 | 124.34 | 52.0 | 23.08 |
| 102 | 264 | 1 | 138.00 | 17.03 | 1.00 | 125.59 | 59.0 | 50.81 |
| 103 | 265 | 1 | 34.00 | 34.30 | 1.00 | 126.82 | 52.0 | 30.66 |
| 104 | 266 | 1 | 42.00 | 30.86 | 1.00 | 128.05 | 52.0 | 35.53 |
| 105 | 267 | 1 | 66.00 | 24.62 | 1.00 | 129.29 | 53.0 | 38.72 |
| 106 | 268 | 1 | 136.00 | 16.01 | 1.00 | 130.54 | 58.0 | 63.75 |
| 107 | 269 | 1 | 81.00 | 22.22 | 1.00 | 131.78 | 55.0 | 33.25 |
| 108 | 270 | 1 | 119.00 | 18.33 | 1.00 | 133.02 | 57.0 | 50.75 |
| 109 | 271 | 1 | 92.00 | 20.85 | 1.00 | 134.25 | 51.0 | 46.79 |
| 110 | 272 | 1 | 168.00 | 15.43 | 1.00 | 135.50 | 59.0 | 52.65 |
| 111 | 273 | 1 | 130.00 | 17.54 | 1.00 | 136.75 | 58.0 | 50.21 |
| 112 | 274 | 1 | 188.00 | 14.59 | 1.00 | 138.00 | 59.0 | 61.70 |
| 113 | 275 | 1 | 45.00 | 29.81 | 1.00 | 139.24 | 51.0 | 34.91 |
| 114 | 276 | 1 | 61.00 | 25.61 | 1.00 | 140.47 | 54.0 | 39.43 |
| 115 | 277 | 1 | 45.00 | 29.81 | 1.00 | 141.70 | 52.0 | 26.28 |
| 116 | 278 | 1 | 134.00 | 16.12 | 1.00 | 142.95 | 60.0 | 61.37 |
| 117 | 279 | 1 | 54.00 | 27.22 | 1.00 | 144.18 | 50.0 | 40.62 |
| 118 | 280 | 1 | 29.00 | 37.14 | 1.00 | 145.41 | 52.0 | 28.05 |
| 119 | 281 | 1 | 99.00 | 20.10 | 1.00 | 146.65 | 56.0 | 42.92 |
| 120 | 282 | 1 | 37.00 | 32.88 | 1.00 | 147.88 | 60.0 | 27.07 |
| 121 | 283 | 1 | 51.00 | 28.01 | 1.00 | 149.12 | 61.0 | 27.62 |
| 122 | 284 | 1 | 63.00 | 25.20 | 1.00 | 150.35 | 57.0 | 23.21 |
| 123 | 285 | 1 | 60.00 | 25.82 | 1.00 | 151.58 | 61.0 | 31.22 |
| 124 | 286 | 1 | 67.00 | 24.43 | 1.00 | 152.82 | 53.0 | 24.78 |
| 125 | 287 | 1 | 65.00 | 24.81 | 1.00 | 154.05 | 56.0 | 29.27 |
| 126 | 288 | 1 | 47.00 | 29.17 | 1.00 | 155.27 | 57.0 | 16.35 |
| 127 | 289 | 1 | 45.00 | 29.81 | 1.00 | 156.51 | 61.0 | 18.46 |
| 128 | 290 | 1 | 59.00 | 26.04 | 1.00 | 157.73 | 56.0 | 13.03 |
| 129 | 291 | 1 | 81.00 | 22.22 | 1.00 | 158.97 | 56.0 | 48.20 |
| 130 | 292 | 1 | 104.00 | 17.61 | 1.00 | 160.21 | 58.0 | 34.80 |
| 131 | 293 | 1 | 77.00 | 22.79 | 1.00 | 161.45 | 55.0 | 42.13 |
| 132 | 294 | 1 | 144.00 | 16.67 | 1.00 | 162.68 | 55.0 | 13.30 |
| 133 | 295 | 1 | 60.00 | 25.82 | 1.00 | 163.91 | 56.0 | 33.86 |
| 134 | 296 | 1 | 53.00 | 27.47 | 1.00 | 165.15 | 61.0 | 32.19 |
| 135 | 297 | 1 | 60.00 | 25.82 | 1.00 | 166.38 | 56.0 | 32.51 |
| 136 | 298 | 1 | 115.00 | 18.65 | 1.00 | 167.62 | 59.0 | 60.08 |
| 137 | 299 | 1 | 102.00 | 19.80 | 1.00 | 168.86 | 61.0 | 58.56 |
| 138 | 1 | 1 | 40.00 | 31.62 | 1.00 | 170.10 | 61.0 | 14.29 |
| 139 | 2 | 1 | 43.00 | 30.50 | 1.00 | 171.33 | 59.0 | 20.39 |
| 140 | 3 | 1 | 34.00 | 34.30 | 1.00 | 172.56 | 62.0 | 28.12 |
| 141 | 4 | 1 | 82.00 | 22.09 | 1.00 | 173.79 | 56.0 | 31.15 |
| 142 | 5 | 1 | 56.00 | 28.28 | 1.00 | 175.03 | 54.0 | 39.19 |
| 143 | 6 | 1 | 72.00 | 23.57 | 1.00 | 176.27 | 55.0 | 57.92 |
| 144 | 7 | 1 | 69.00 | 24.08 | 1.00 | 177.49 | 54.0 | 25.80 |
| 145 | 8 | 1 | 93.00 | 20.74 | 1.00 | 178.73 | 60.0 | 41.00 |
| 146 | 9 | 1 | 47.00 | 29.17 | 1.00 | 179.97 | 55.0 | 31.18 |
| 147 | 10 | 1 | 44.00 | 30.51 | 1.00 | 181.20 | 55.0 | 42.84 |
| 148 | 11 | 1 | 57.00 | 26.49 | 1.00 | 182.43 | 54.0 | 39.87 |
| 149 | 12 | 1 | 36.00 | 33.33 | 1.00 | 183.67 | 55.0 | 44.03 |
| 150 | 13 | 1 | 57.00 | 26.49 | 1.00 | 184.89 | 58.0 | 14.23 |
| 151 | 14 | 1 | 38.00 | 32.44 | 1.00 | 186.12 | 62.0 | 20.38 |
| 152 | 15 | 1 | 45.00 | 29.81 | 1.00 | 187.35 | 61.0 | 19.47 |
| 153 | 16 | 1 | 212.00 | 13.74 | 1.00 | 188.60 | 60.0 | 39.16 |
| 154 | 17 | 1 | 60.00 | 25.82 | 1.00 | 189.83 | 52.0 | 25.99 |
| 155 | 18 | 1 | 88.00 | 21.32 | 1.00 | 191.06 | 54.0 | 33.97 |
| 156 | 19 | 1 | 81.00 | 22.22 | 1.00 | 192.30 | 55.0 | 26.85 |
| 157 | 20 | 1 | 131.00 | 17.47 | 1.00 | 193.55 | 59.0 | 58.40 |
| 158 | 21 | 1 | 61.00 | 25.61 | 1.00 | 194.78 | 60.0 | 56.90 |
| 159 | 22 | 1 | 52.00 | 27.74 | 1.00 | 196.02 | 57.0 | 72.44 |
| 160 | 23 | 1 | 42.00 | 30.86 | 1.00 | 197.25 | 54.0 | 24.32 |

Handwritten margin labels (left): T-F5, EDAP, EL, E3, E4, E5, bf, bf4

3/8/96   induction of recombinant proteins



HTPA (Trail-long) / pQE9



LPA (Trail-short) / pQE

BamHI          HinDIII    oligos

His-tagged



Figure 1. Nucleotide and Predicted Amino Acid Sequence of the TRAIL cDNA

The cDNA sequence of the human TRAIL gene is shown. Numbers at the right indicate the nucleotide number (top) and amino acid number (bottom). Amino acids comprising the predicted transmembrane region are underlined. Underlined nucleotides near the 3' end form a consensus polyadenylation signal.

TNF-α expression in pQE-9    His tagged



HP00012/pQE9



CS46    CS47
#14    #15    #16    #17

8.

3/12/96    TNF Receptor like    HTTBN61
Now called Death Domain containing receptor DDCR

Fulllength clone HLMH058 contained a stop codon

after methionine



M                open reading frame

stop    potential signal peptide
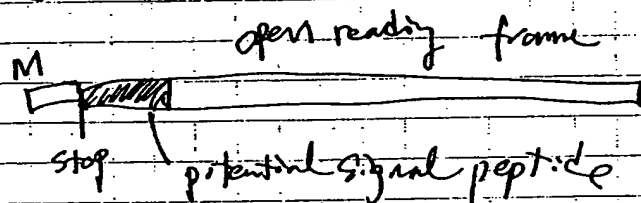
the stop codon is real : with many rounds of sequencing

① to get New clones

② PCR 5' seq direct sequence

oligo HTTBN61Rp10 made

CCCCTCCGAAGCCTGGTGT



M

Rp10    RpR1 RpR2
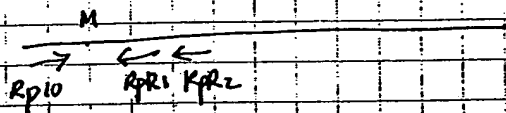
use Rp10 and RpR2 to PCR libraries. Sequence

the product with RpR1

3/13/98  Expression vectors:

① EDAP Δ2       5' CGCCCATGGCCTTCACCAAGAACCGA NcoI
        d48

② EDAP-Δ3       5' CGCCCATGGACTATACCAACAAATTC NcoI
        d54

both clone into pQE60      3' will use EDAP Hind III

③ AIM2 Δ1  5' CGCCCATGGCCAACTCCAGCTTGACC NcoI

④ AIM2 Δ2  5' CGCCCATGGTCACCCGCCTGCCTGAG NcoI

both clone into pQE60      3' Hind III

SUPERVISOR:
DATE:
SUPERVISOR:
DATE:

CSG16/pREb0

3/19/86 Nwg

CSG



HT4CC12/pREb0

#5  #6  #7  #8

3/15/9 b
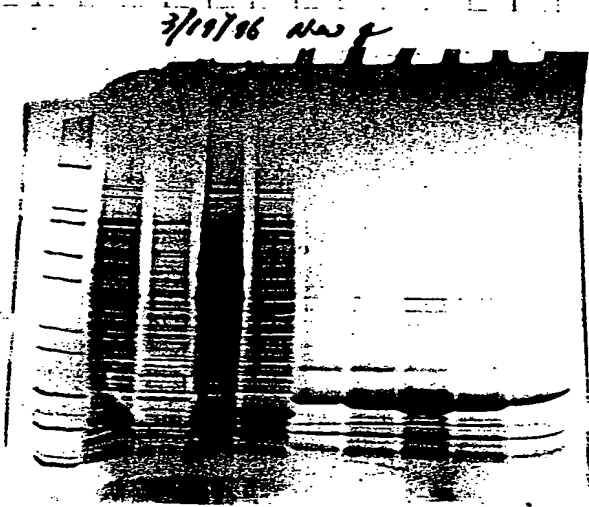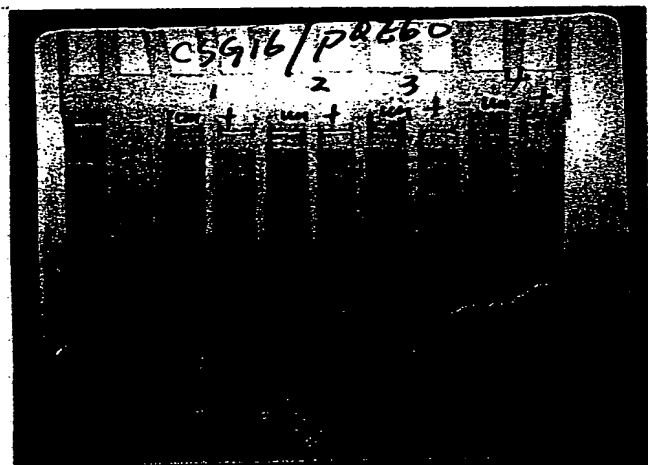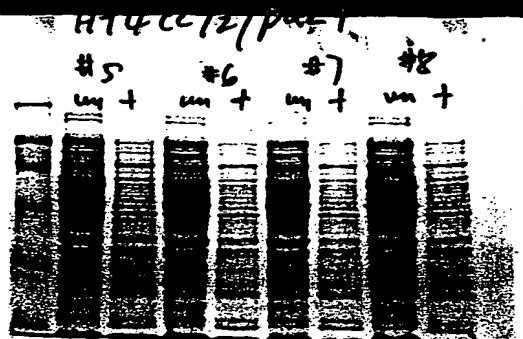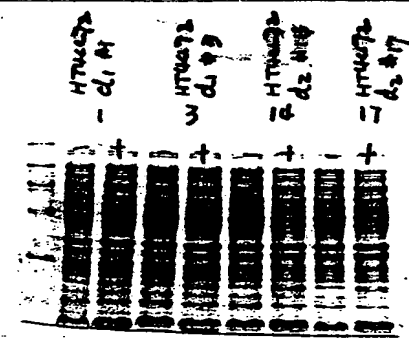
all gene in pREb0

10% SDS
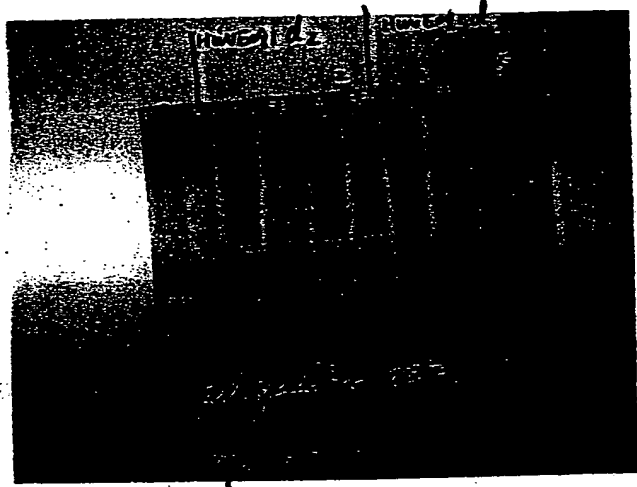
# EDAP Expression vector position

```
 781  ATATGAGACGCTTTTTAAGCAAAGTCTACAGTTTCCCAATGAGAAAATTAATCCTCTTTC
         M   R   R   F   L   S   K   V   Y   S   F   P   M   R   K   L   I   L   F   L
       PQE9,  PQE70,  CHO
 841  TTGTCTTTCCAGTTGTGAGACAAACTCCCACACAGCACTTTAAAAATCAGTTCCCAGCTC
         V   F   P   V   V   R   Q   T   P   T   Q   H   F   K   N   Q   F   P   A   L
         IL6      PQE60,  pA2-GP
 901  TGCACTGGGAACATGAACTAGGCCTGGCCTTCACCAAGAACCGAATGAACTATACCAACA     PQE60
         H   W   E   H   E   L   G   L   A   F   T   K   N   R   M   N   Y   T   N   K
 961  AATTCCTGCTGATCCCAGAGTCGGGAGACTACTTCATTTACTCCCAGGTCACATTCCGTG
         F   L   L   I   P   E   S   G   D   Y   F   I   Y   S   Q   V   T   F   R   G
1021  GGATGACCTCTGAGTGCAGTGAAATCAGACAAGCAGGCCGACCAAACAAGCCAGACTCCA
         M   T   S   E   C   S   E   I   R   Q   A   G   R   P   N   K   P   D   S   I
1081  TCACTGTGGTCATCACCAAGGTAACAGACAGCTACCCTGAGCCCAACCCAGCTCCTCATGG
         T   V   V   I   T   K   V   T   D   S   Y   P   E   P   T   Q   L   L   M   G
1141  GGACCAAGTCTGTATGCGAAGTAGGTAGCAACTGGTTCCAGCCCATCTACCTCGGAGCCA
         T   K   S   V   C   E   V   G   S   N   W   F   Q   P   I   Y   L   G   A   M
1201  TGTTCTCCTTGCAAGAAGGGGACAAGCTAATGGTGAACGTCAGTGACATCTCTTTGGTGG
         F   S   L   Q   E   G   D   K   L   M   V   N   V   S   D   I   S   L   V   D
1261  ATTACACAAAAGAAGATAAAAACCTTCTTTGGAGCCTTCTTACTATAGGAGGAGAGCAAAT
         Y   T   K   E   D   K   T   F   F   G   A   F   L   L   *
```

838 d₁

448 d₂

454 d₃